Listing of Claims

1. (Currently Amended) A method of creating a clinical reference solution that emulates clinically relevant sites on genes responsible for human genetic conditions, wherein the clinical reference solution is substantially free of clinically irrelevant nucleic acid, comprising:

for each clinically relevant site, designing an oligonucleotide comprising an arrangement of bases to emulate the clinically relevant site as isolated from clinically irrelevant nucleic acid that occurs adjacent to the corresponding clinically relevant site in vivo, ~~wherein~~ including designing two ends of the arrangement ~~of bases also~~ to form[[s]] ~~one or more~~ primer targets for differentially amplifying the emulated clinically relevant site;

for each ~~clinically relevant site~~ arrangement, performing a non-ligase, non-template, non-cloning synthesis ~~synthesizing~~ that includes constructing base-by-base, from end to end, a single strand of bases comprising the arrangement of bases that emulates the clinically relevant site and forms the primer targets associated with the clinically relevant site; and

mixing each single strand into a single solution to form a collection of oligonucleotides each representing a clinically relevant site of a gene.

2. (Previously Presented) The method as recited in claim 1, wherein each clinically relevant site comprises a mutation of a normal human nucleic acid sequence, each mutation representing a human genetic condition.

3. (Canceled)

4. (Currently Amended) The method as recited in claim 1, wherein:

the synthesizing the single strand includes constructing a first sequence of nucleotides attached base-by-base to a first end of the arrangements of bases, wherein the first sequence is complementary to a nucleotide sequence of a first primer of a primer set, and

the synthesizing the single strand includes constructing a second sequence of nucleotides attached base-by-base to a second end of the arrangements of bases, wherein the second sequence is identical to a nucleotide sequence of a second primer of a primer set.

5. (Currently Amended) The method as recited in claim 1, wherein the synthesizing comprises constructing ~~synthesizing~~, base-by-base, two complementary strands, wherein:

a first strand includes one of the clinically relevant sites and a nucleic acid tag complementary to a first primer of a primer set; and

a second strand is complementary to the first strand and to a nucleic acid tag complementary to a second primer of the primer set.

6-7. (Canceled)

8. (Currently Amended) The method as recited in claim 1, wherein:

each emulated clinically relevant site has an associated primer set, and wherein:

the reference solution is tuned for a specific battery of clinical tests by differentially amplifying the different emulated clinically relevant sites to different concentrations in the reference solution.

9. (Currently Amended) The method as recited in claim 8, wherein different groups of the emulated clinically relevant sites in the reference solution have associated primer sets such that each different group of emulated clinically relevant sites is amplified independently.

10. (Canceled)

11. (Currently Amended) The method as recited in claim 9, wherein independently amplifying each of the groups of emulated clinically relevant sites includes controlling a physical characteristic of the reference

solution to favor an amplification capability of one primer set over an amplification capability another primer set.

12-15. (Canceled)

16. (Currently Amended) The method as recited in claim 1, further comprising adding normal human nucleic acid to the collection of oligonucleotides ~~base-by-base synthesized clinically relevant sites~~ in order to achieve a mixture of the nucleic acids in the reference solution representing at least a segment of homologous heterozygous alleles.

17-20. (Canceled)

21. (Previously Presented) The method as recited in claim 1, further comprising joining two parts of one of the arrangements of bases together using a ligation extension to perform the synthesizing of a large arrangement of bases.

22. (Previously Presented) The method as recited in claim 21, further comprising using a bridge nucleic acid to join multiple parts of the arrangement of bases.

23. (Previously Presented) The method as recited in claim 1, further comprising using an overlap extension to join multiple parts of the arrangement of bases.

24-70. (Canceled)

71. (Currently Amended) A method for tuning concentrations of different reference nucleic acids within a clinical reference solution, comprising:

designing multiple reference nucleic acids, wherein each reference nucleic acid comprises an arrangement of bases emulating a clinically relevant site on genes responsible for human genetic conditions ~~of a human nucleic acid~~ exclusive of clinically irrelevant human nucleic acid adjacent to the clinically relevant site in vivo;

~~synthesizing, base-by-base~~ for each reference nucleic acid [[,]] in a first subset of the multiple reference nucleic acids, constructing an oligonucleotide comprising an arrangement of bases to emulate the clinically relevant site as isolated from clinically irrelevant nucleic acid that occurs adjacent to the corresponding clinically relevant site in vivo, including designing two ends of the arrangement to form a first pair of primer targets ~~a first mixture of various of the reference nucleic acids, wherein each of the various reference nucleic acids in the first mixture includes one or more tags~~ allowing PCR amplification of the first subset

~~mixture~~ via a primer set specific to the first pair of primer targets ~~tags of the first mixture~~; ~~and~~

~~synthesizing, base-by-base~~ for each reference nucleic acid [[,]]in a second subset of the multiple reference nucleic acids, constructing an oligonucleotide comprising an arrangement of bases to emulate the clinically relevant site as isolated from clinically irrelevant nucleic acid that occurs adjacent to the corresponding clinically relevant site in vivo, including designing two ends of the arrangement to form a second pair of primer targets ~~a second mixture of various of the reference nucleic acids, wherein each of the various reference nucleic acids in the second mixture includes one or more tags~~ allowing PCR amplification of the second subset ~~mixture~~ via a second primer set specific to the second pair of primer targets ~~tags of the second mixture~~; and

wherein each oligonucleotide in the first and second subsets is constructed base-by-base, from end to end, as a single strand via a non-ligase, non-template, non-cloning synthesis.

72. (Currently Amended) The method as recited in claim 71, further comprising combining the first and second subsets ~~mixtures~~ to make a single mixture and differentially amplifying the first subset ~~mixture~~ and the second subset ~~mixture~~ in a PCR reaction by controlling amounts of the first primer set and the second primer set in the single mixture.

73. (Canceled)

74. (Previously Presented) The method as recited in claim 72, further comprising adding normal human nucleic acid to the single mixture to obtain heterozygous pairs, wherein each heterozygous pair includes a normal segment of human nucleic acid and a mutated copy of the normal segment of human nucleic acid.

75. (Currently Amended) The method as recited in claim 1, further comprising differentially amplifying each different type of oligonucleotide ~~single strand~~ in the single solution to a respective clinically relevant concentration.

76. (Previously Presented) The method as recited in claim 75, wherein the amplifying increases the number of each single strand exponentially.

77. (Previously Presented) The method as recited in claim 75, wherein each single strand creates its own complementary single strand during the amplifying.

78-80. (Canceled)